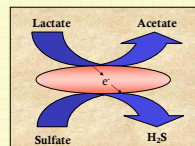


## ABSTRACT

A sulfate reducing bacterium was isolated from the Paleta Creek site in San Diego Bay with 60mM lactate as the sole carbon source and electron donor, and 50mM sulfate as the electron acceptor. The novel isolate, strain PCS is an anaerobic, non-sporulating, gram-negative organism that is highly motile. The optimum temperature for growth of strain PCS was determined to be 37 °C. Preliminary 16S rDNA analysis revealed that the closest relative to strain PCS is *Desulfovibrio africanus* (98 % similar). Light microscopy and SEM images of individual cells reveal sigmoid morphology. Cells of strain PCS appear like slender curved rods during the early log phase and spiral in exponential /stationary phase to approx 5-10µm in length and 0.3µm in width. In this regard, strain PCS is less than half the width of its closest known relative *D.africanus*. The images also reveal the presence of lemon shaped/spherical structures approx 1µm in diameter especially in early log and stationary phases of growth. Detailed investigations are underway to determine the nature and function of these structures. When grown on LS4D minimal media, strain PCS incompletely oxidizes lactate, accumulating acetate as an end product. Sulfate is reduced to hydrogen sulfide. Apart from lactate, strain PCS also utilizes alternative electron donors like pyruvate, benzoate and dihydroxyacetone. Propionate, butyrate and formate were not utilized. Alternative electron acceptors utilized includes Fe-NTA and thiosulfate. Nitrate and chlorate were not reduced. When tested for reduction of toxic metal like Cr(VI), a washed cell suspension of strain PCS could remove almost 150 µM of Cr(VI) supplied as potassium chromate with lactate as the electron donor. This high chromium reducing capability of strain PCS is of great significance for the potential utilization of this microbe towards the treatment of Cr(VI) contaminated environments.

## ISOLATION AND MORPHOLOGY



Strain PCS was isolated on anaerobic LS4D medium with 60mM lactate as the sole carbon source and electron donor and 50mM sulfate as the electron acceptor from sediment collected (courtesy Dr. J. D. Coates) from Paleta Creek site in San Diego Bay. Colonies of strain PCS take 3 weeks to appear on solid agar. Microscopic observation of strain PCS revealed pleomorphic nature of the organism.

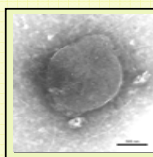
Strain PCS appeared mostly as long thin sigmoid/spiral cells during exponential phase of growth. During early log/stationary phase, lemon shaped cells predominate.

The lemon shaped structures were not spores and were DNA containing bodies as determined by picogreen staining.

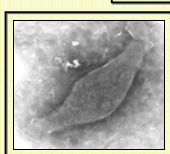
SEM



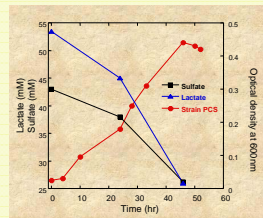
Individual cells of sigmoid/spiral morphology measure 5-10µm in length and 0.5µm in width. Cells are extremely motile with lophotrichous arrangement of flagella. The lemon shaped cells are approx 1µm wide and 1.2µm in length.



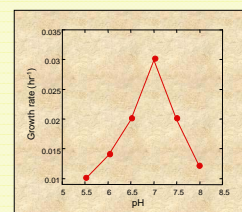
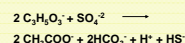
PIA stained TEM



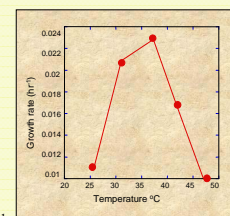
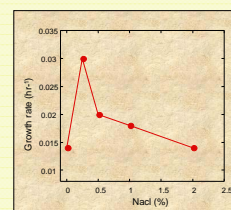
## CHARACTERIZATION



When growing on LS4D media, strain PCS reached stationary phase after 48 hours incubation at 30 °C. At this time, 29mM lactate had been oxidized and 16.4 mM sulfate reduced to give a stoichiometry of 96% of the theoretical value.

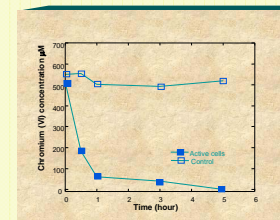


The optimum temperature for strain PCS was determined to be 37 °C. Although the optimum salinity was 0.3%, strain PCS could survive in media containing upto 5% NaCl. Strain PCS could grow over a pH range from 5.5 to 8, the optimal pH for growth being circumneutral.



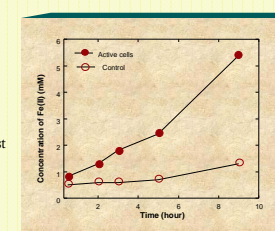
Electron acceptor	Result
Sulfate	+
Thiosulfate	+
Sulfite	+
Sulfur	-
Nitrate	-
Fumarate	-
Chromium(VI)	+
Fe(III)	+
Chlorate	-
Oxygen	-

## METAL REDUCTION



An active washed cell suspension of strain PCS enzymatically reduced 500 µM Chromium (VI) supplied as potassium chromate within 5 hours with lactate as the electron donor. No reduction occurred in parallel incubations without electron donor. Further, in the absence of cells, no abiotic reduction of Cr(VI) took place (data not shown).

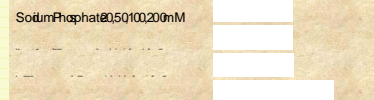
Similar to Cr(VI), active cells of strain PCS also enzymatically reduced Fe(III) supplied as Ferric citrate to Fe(II). Almost 5mM Fe(III) was reduced over 9 hours relative to controls. Lactate served as the electron donor in this experiment.



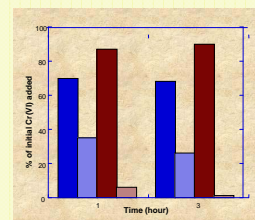
## Osmotic sensitivity



## Toxicity Tests



The Cr(VI) reducing capability of nitrate stressed *D. vulgaris* strain Hildenborough was compared to that of nitrate stressed cells of strain PCS. Preliminary results suggest that strain PCS can reduce hexavalent chromium more efficiently than strain DvH. The results also suggest that both these strains could successfully reduce hexavalent chromium even under nitrate stress. This result is encouraging in devising bioremediative strategies for Cr(VI) reduction by sulfate reducing bacteria co-contaminated with nitrate.



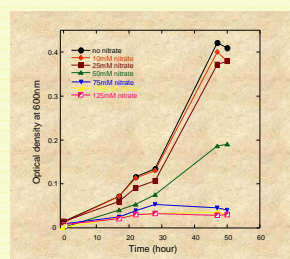
## CONCLUSION

- Strain PCS is a gram negative, highly motile, non spore forming, pleomorphic, sulfate reducing delta-Proteobacteria isolated from hydrocarbon impacted sediments of San Diego Bay.
- Strain PCS can utilize a wider range of organic acids and sugars as its C-source compared to its closest relative *D. africanus*.
- While originally isolated and maintained on fresh water medium, strain PCS exhibits wide salt tolerance of upto 5% NaCl and 6% KCl.
- Strain PCS can enzymatically reduce high concentration of toxic, soluble chromate to its non-toxic form.
- Cr(VI) reducing capability of strain PCS is not diminished under stress conditions.

## ACKNOWLEDGEMENTS

This work was part of the Virtual Institute for Microbial Stress and Survival supported by the U. S. Department of Energy, Office of Science, Office of Biological and Environmental Research, Genomics Program/GTL through contract DE-AC03-76SF00098 between Lawrence Berkeley National Laboratory and the U. S. Department of Energy.

The OMNILOG phenotypic microarray was used to study substrate utilization, osmotic sensitivity and toxicity tests. Since the optimum temperature for strain PCS was determined to be 37 °C, the OMNILOG was operated at that temperature using LS4D medium as well as modified Baar's medium for better reproducibility.



The effect of nitrate on the growth of strain PCS was studied in LS4D medium with lactate as the electron donor and sulfate as the electron acceptor. While 10mM and 25mM nitrate had almost no effect, 50mM nitrate inhibited cell growth considerably. For further studies on strain PCS under nitrate stress, this concentration of nitrate was used.